Forensic Genetics Symposium
Organized with the Rio de Janeiro Court of Justice
Rio de Janeiro, Brazil
5 May 2017

IV Simpósio Internacional de Identificação Humana por DNA - Rio de Janeiro

Current Challenges Facing Forensic Genetics

John M. Butler, Ph.D.

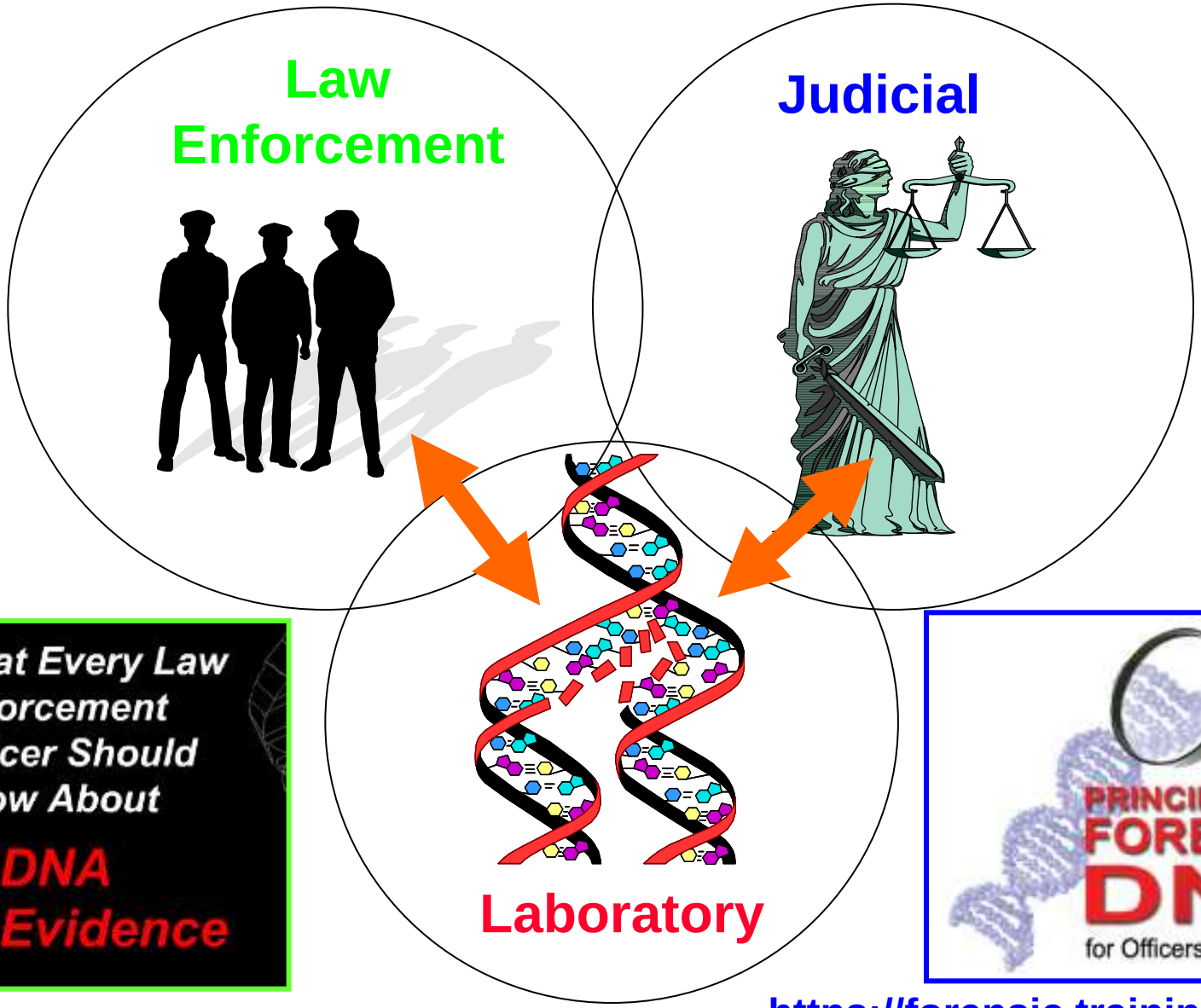
NIST Fellow & Special Assistant to the Director for Forensic Science

U.S. National Institute of Standards and Technology

Talk presented by Dr. Mecki Prinz

John Jay College of Criminal Justice,
New York City, United States of America

Communication Across the Criminal Justice System is Important



What Every Law Enforcement Officer Should Know About

DNA Evidence

PRINCIPLES OF FORENSIC DNA for Officers of the Court

https://forensic.training.nij.gov/

NATIONAL INSTITUTE OF JUSTICE

TRAINING: *Forensic DNA for Officers of the Court*

Home | Glossary | Resources

https://forensic.training.nij.gov/

- + 01 Introduction
- + 02 Biology of DNA
- + 03 Practical Issues Specific to DNA Evidence
- + 04 Introduction to the Forensic DNA Laboratory
- + 05 Assuring Quality in DNA Testing
- + 06 Understanding a Forensic DNA Lab Report
- + 07 Statistics and Population Genetics
- + 08 Mitochondrial DNA & Y-STR Analysis
- + 09 Forensic DNA Databases
- + 10 Collection of DNA Evidence from Suspects and Arrestees
- + 11 Pretrial DNA Evidence Issues
- + 12 Victim Issues
- + 13 Trial Presentation
- + 14 Postconviction DNA Cases
- + 15 Emerging Trends

Login

Security Upgrades

You should already have noticed and agreed to a new disclaim
made to make our registration process and courses more secu

- Your password must now be at least **8** characters and inc
  letters, numbers, and special characters (e.g., ~!@&#$%^
- You will be asked to reset your password every **90** days.
- You may not reuse any of your past **6** passwords.

Please Login

Username

Password

Login

Don't have an account? Register Now

Forgot your username? Retrieve your username now.

Butler Books on Forensic DNA Typing

National Institute of Standards and Technology

- Science agency **part of the U.S. Department of Commerce**
- Started in 1901 as the **National Bureau of Standards**
- Name changed in 1988 to the **National Institute of Standards and Technology (NIST)**
- Forensic science research activities dating back to 1920s
- Partnership since 2013 with U.S. Department of Justice to create the National Commission on Forensic Science (NCFS) and the Organization of Scientific Area Committees (OSAC)

- Primary campus in Gaithersburg, Maryland (near Washington, D.C.)
- >3,400 employees and >3,700 associates
- Supplies >1300 reference materials
- Defines official time for the U.S.

DNA reference material

NIST Forensic Science Efforts

Partnership with Department of Justice

National Commission on Forensic Science (NCFS)

Charter completed on April 23, 2017

Department of Justice FACA co-led by NIST
setting policy

Organization of Scientific Area Committees (OSAC)

NIST-administered
>540 members of the community
establishing standards and best practices

Assessing scientific foundations and method validation for select forensic disciplines

NIST Funded Internal Research Programs

NIST FORENSIC SCIENCES

~$7.5M/year invested

NIST Forensic Science Center of Excellence

IOWA STATE

CoE: ~$4M/year invested for 5 years (2015-2020)

International Symposium on Forensic Science Error Management

432 participants (11 countries)

Forensic Conference Organized by NIST

Planning has started for a second Symposium
Date: July 24-28, 2017
Location: Gaithersburg, MD
Sponsors that have been approached
DoD, FBI, NIST

http://www.nist.gov/director/international_forensics_home.cfm

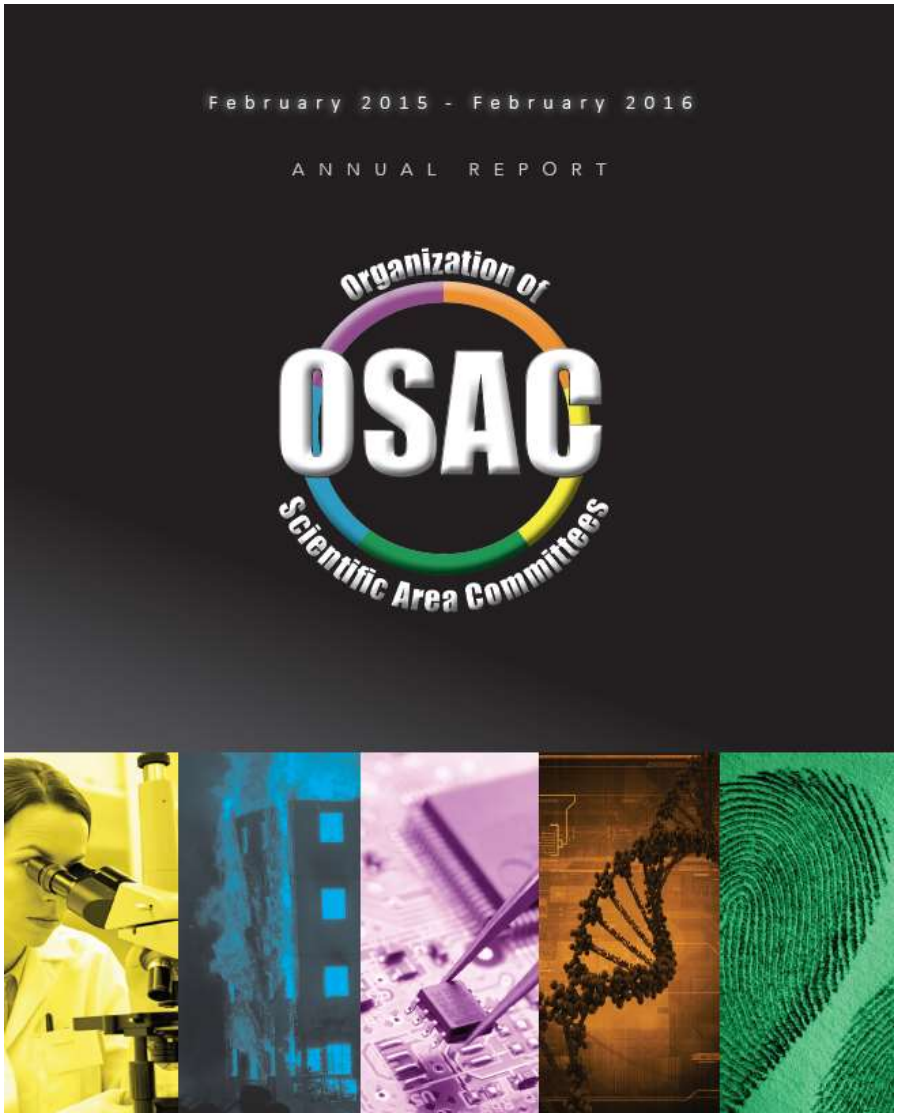
National Institute of Standards and Technology
U.S. Department of Commerce

Organization of OSAC Scientific Area Committees for Forensic Science

>600 people involved in 34 operational units
http://www.nist.gov/forensics/osac/index.cfm

- Provides technical leadership to help develop and promulgate **consensus-based documentary standards and guidelines** for forensic science
- Promotes standards and guidelines that are **fit-for-purpose** and **based on sound scientific principles**
- Promotes the use of OSAC documents by accreditation and certification bodies
- Establishes and maintains working relationships with similar organizations

OSAC Annual Report



- **74 page report** summarizing activities from the first year of OSAC (Feb 2015 to Feb 2016)

- Available as a pdf file for download at https://www.nist.gov/sites/default/files/documents/2016/09/13/osac_annual_report_2015-2016.pdf

Released 19 September 2016

DNA Capabilities to Aid Forensic Investigations

1. The **ability to identify the perpetrator**
2. Weight-of-evidence based on established genetic principles and statistics (Hardy-Weinberg 1908)
3. Established characteristics of genetic inheritance enables close **biological relatives** to be used for reference points using kinship associations
4. Superb **sensitivity** with PCR amplification (opens the possibility for contamination)
5. Well-established **quality assurance measures**
6. New **technology development** aided by genomics

Successful interpretation of DNA (Q-to-K comparison) depends on quality of the crime scene evidence (Q) and availability of suitable reference samples (K)

Professor Peter Gill

June 2014; 100 pages

Concerns have been Raised over Potential for DNA Contamination

Previous articles by Peter Gill on this topic:

- Gill, P. (1997). The utility of 'substrate controls' in relation to 'contamination'. *Forensic Science International,* 85(2):105-111.
- Gill, P., & Kirkham, A. (2004). Development of a simulation model to assess the impact of contamination in casework using STRs. *Journal of Forensic Sciences,* 49(3): 485-491.
- Gill, P., et al. (2010). Manufacturer contamination of disposable plastic-ware and other reagents—an agreed position statement by ENFSI, SWGDAM and BSAG. *Forensic Science International: Genetics,* 4(4): 269-270.

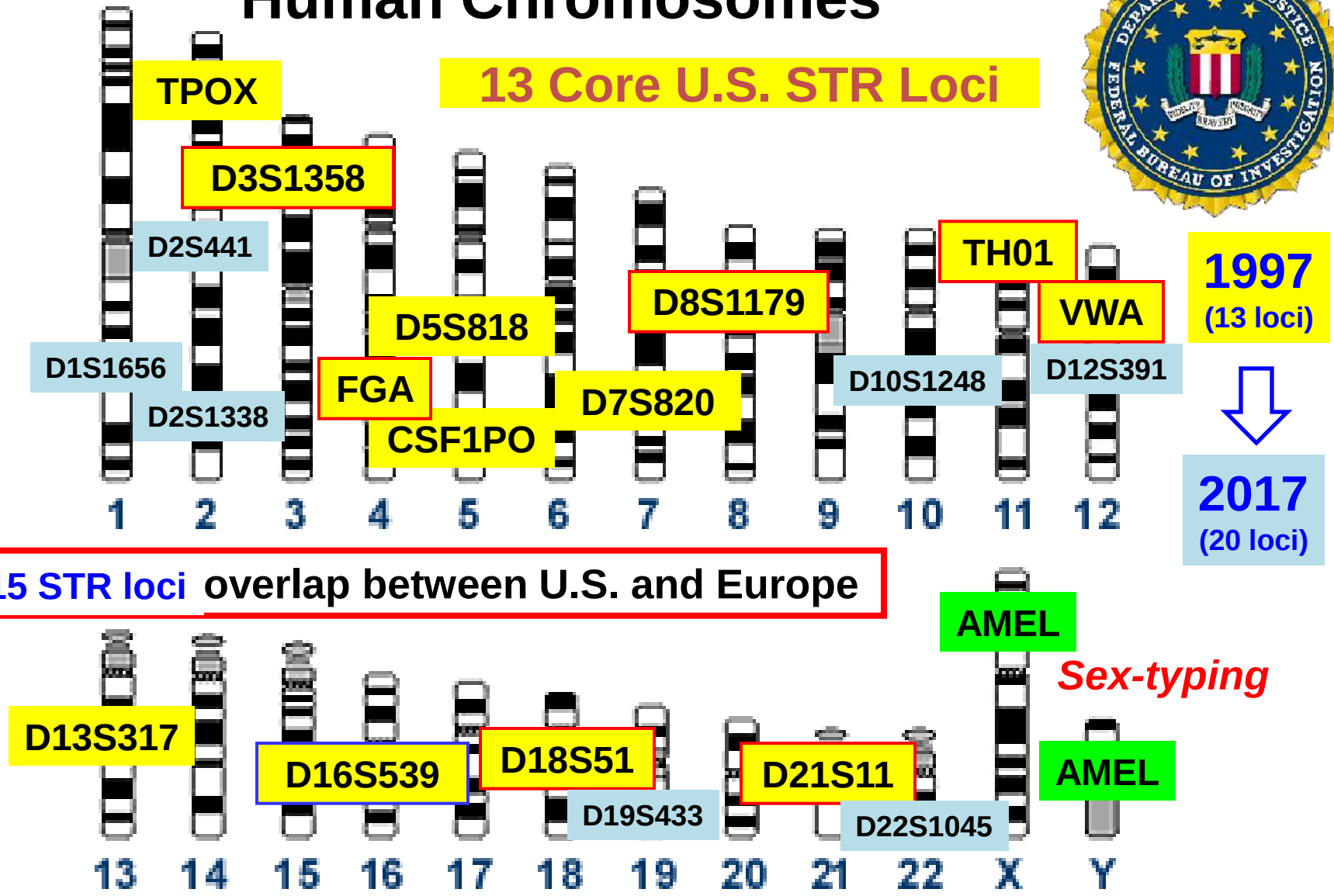
Discusses the Amanda Knox case DNA results

Forensic DNA Testing in the United States

- We have **~200 public (state and local government) laboratories** performing forensic DNA analysis
  - Two large private companies (Bode Cellmark and Sorenson Forensics) and a few smaller ones perform forensic DNA analysis
- Over 15 million DNA profiles in the national DNA database (NDIS: National DNA Index System) run by the FBI Laboratory
  - Since 1998, the U.S. has included 13 core STR (short tandem repeat) markers; starting in 2017, this number has increased to 20 required STR loci
- Laboratories have many different protocols and in some cases, submitting the same sample to two different laboratories could result in two different results
  - Efforts are underway to improve standardization in the field

Position of Forensic STR Markers on Human Chromosomes

Core STR Loci for the United States



Checks and Controls on Forensic DNA Results

Community	FBI DNA Advisory Board's Quality Assurance Standards *(also interlaboratory studies)*
Laboratory	ASCLD/LAB, ANAB, A2LA Audits and Accreditation
Analyst	Proficiency Tests & Continuing Education
Method/Instrument	**Validation of Analytical Performance** *(with aid of **traceable reference materials**)*
Protocol	Standard Operating Procedure is followed
Data Sets	Allelic ladders, positive and negative amplification controls, and reagent blanks are used
Individual Sample	Internal size standard present in every sample
Interpretation of Result	**Second review by qualified analyst/supervisor**
Court Presentation of Evidence	Defense attorneys and experts with power of discovery requests

Thoughts on the Future of Forensic DNA Published in 2015

PHILOSOPHICAL TRANSACTIONS B

rstb.royalsocietypublishing.org

Opinion piece

Cite this article: Butler JM. 2015 The future of forensic DNA analysis. *Phil. Trans. R. Soc. B* **370**: 20140252.
http://dx.doi.org/10.1098/rstb.2014.0252

Accepted: 26 February 2015

One contribution of 15 to a discussion meeting issue 'The paradigm shift for UK forensic science'.

The future of forensic DNA analysis

John M. Butler

National Institute of Standards and Technology, Gaithersburg, MD, USA

The author's thoughts and opinions on where the field of forensic DNA testing is headed for the next decade are provided in the context of where the field has come over the past 30 years. Similar to the Olympic motto of 'faster, higher, stronger', forensic DNA protocols can be expected to become more rapid and sensitive and provide stronger investigative potential. New short tandem repeat (STR) loci have expanded the core set of genetic markers used for human identification in Europe and the USA. Rapid DNA testing is on the verge of enabling new applications. Next-generation sequencing has the potential to provide greater depth of coverage for information on STR alleles. Familial DNA searching has expanded capabilities of DNA databases in parts of the world where it is allowed. Challenges and opportunities that will impact the future of forensic DNA are explored including the need for education and training to improve interpretation of complex DNA profiles.

Addressed Rapid DNA and Next-Generation Sequencing

Butler, J.M. (2015) The future of forensic DNA analysis. *Phil. Trans. R. Soc.* B 370: 20140252

Stages of Forensic DNA Progression

Stages	Time Frame	Description
Exploration	1985 - 1995	Beginnings, different methods tried (RFLP and early PCR)
Stabilization	1995 - 2005	Standardization to STRs, selection of core loci, implementation of Quality Assurance Standards
Growth	2005 - 2015	Rapid growth of DNA databases, extended applications pursued
Sophistication	*2015 to 2025 and beyond*	*Expanding tools available,* ***confronting privacy concerns***

Table 1 from J.M. Butler (2015) The future of forensic DNA analysis. *Phil. Trans. R. Soc. B* 370: 20140252

Critical Challenges Faced Today

- **Success of DNA testing** → significant growth in sample submissions → sample backlogs
  - Laboratory automation and expert system data review
  - Restrictive case acceptance policies to avoid law enforcement investigator 'swab-athons' at crime scenes

- **Greater detection sensitivity** → more complex DNA mixtures and low-template DNA with 'touch' evidence
  - Probabilistic genotyping to cope with increase in data interpretation uncertainty
  - Use of a complexity threshold to avoid "skating on thin ice"

Butler, J.M. (2015) The future of forensic DNA analysis. *Phil. Trans. R. Soc.* B 370: 20140252

Going Beyond the Core Competencies of Forensic DNA Testing…

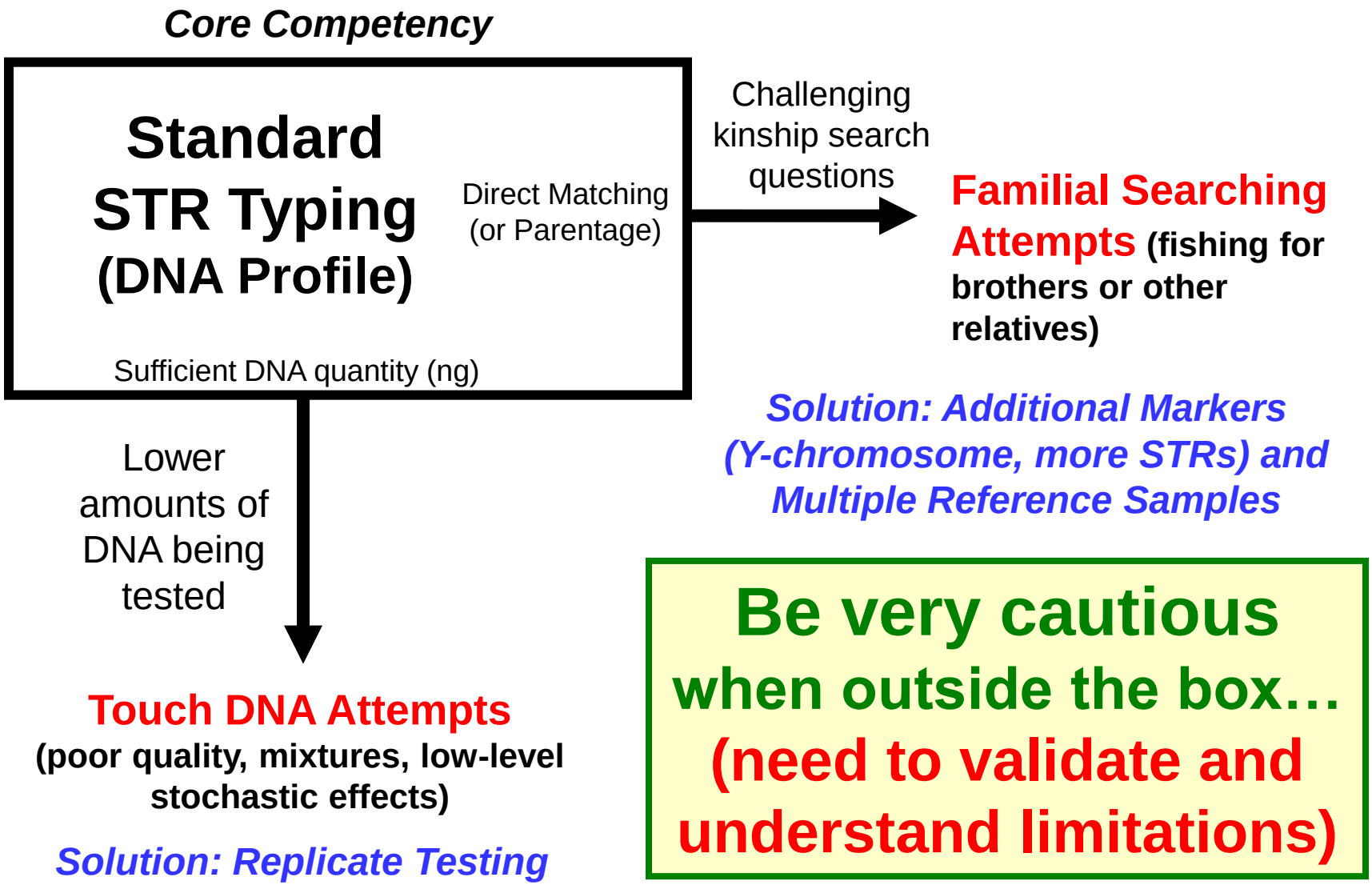


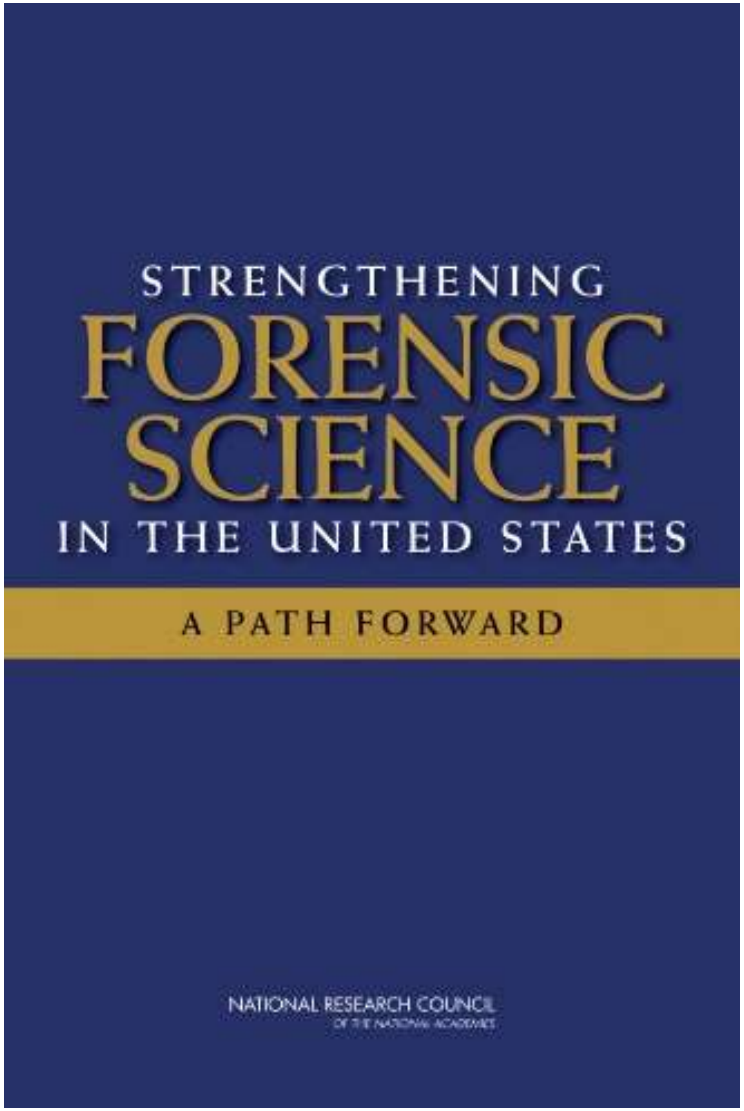
Figure 18.3 in Butler (2010) *Fundamentals of Forensic DNA Typing*

Landmark Report Gives DNA Testing a Pass

Released February 18, 2009

The U.S. National Research Council of the National Academies issued a major report on forensic science in Feb. 2009.

*"**With the exception of nuclear DNA analysis**, no forensic method has been rigorously shown to have the capacity to consistently, and with a high degree of certainty, demonstrate a connection between evidence and a specific individual or source." (p. 41)*



p. 100 mentions limitations with DNA mixtures

PCAST Report Comments on Forensic DNA

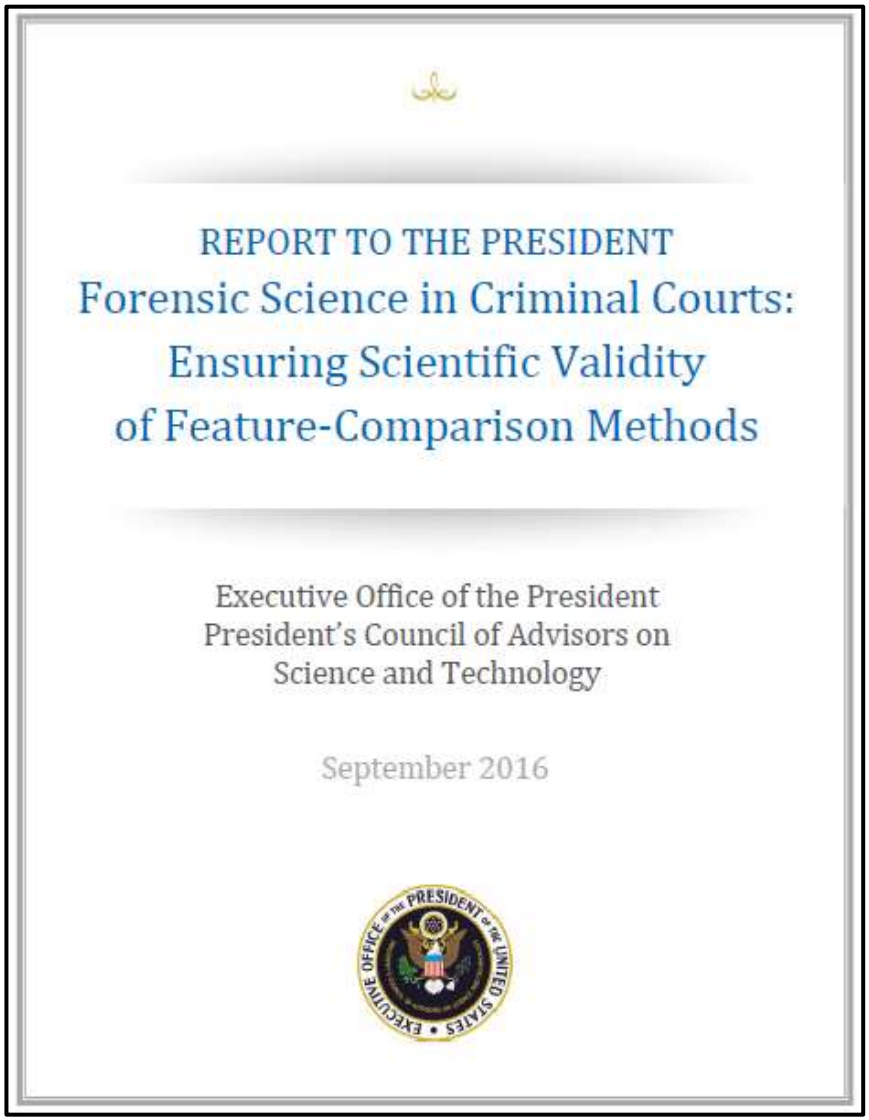
- Supports appropriate use of single-source and simple mixture DNA analysis
- **Expresses reservations with complex DNA mixtures** (≥3 contributors)

PCAST Co-Chairs

Eric Lander

John Holdren

Released September 20, 2016



REPORT TO THE PRESIDENT

Forensic Science in Criminal Courts:
Ensuring Scientific Validity
of Feature-Comparison Methods

Executive Office of the President
President's Council of Advisors on
Science and Technology

September 2016

International conference

The hidden side of DNA profiles. Artifacts, errors and uncertain evidence

Auditorium, Università Cattolica del Sacro Cuore

Rome, 27-28 April, 2012

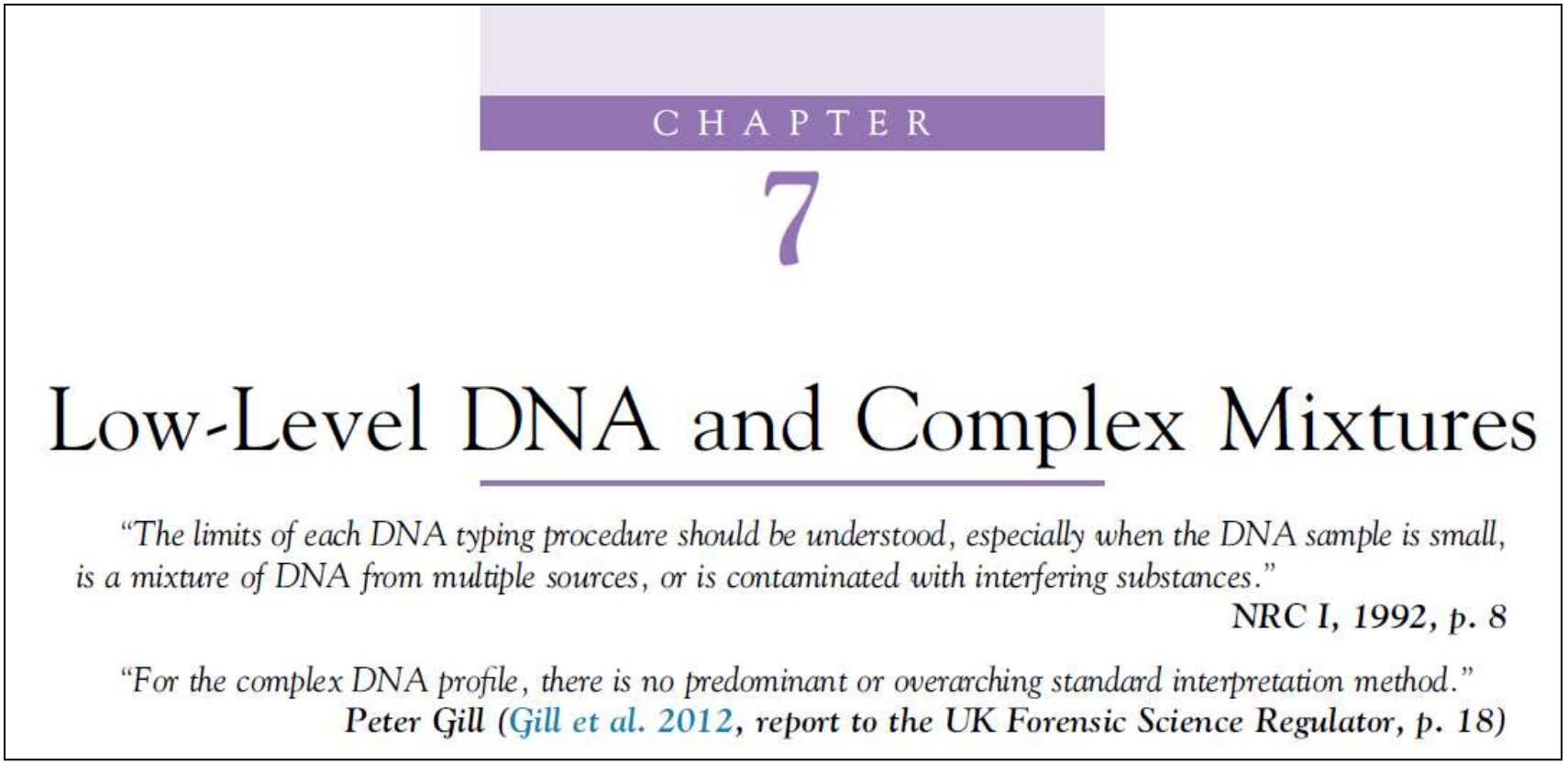
UNIVERSITÀ CATTOLICA del Sacro Cuore

David Balding: “Low-template DNA cases are coming to court with limited abilities for sound interpretation. ... There are dangers with LTDNA but we know how to handle and manage them. Unfortunately, proper management is not a universal practice.”

Peter Schneider: “If you cannot explain your evidence to someone that is not from the field (like a judge) – and you need a lot of technical excuses to report something – then the result is not good. You should leave it on your desk and not take it to court. This is a very common sense approach to this problem.”

Information from Chapter 7

Advanced Topics in Forensic DNA Typing: Interpretation



CHAPTER

7

Low-Level DNA and Complex Mixtures

"The limits of each DNA typing procedure should be understood, especially when the DNA sample is small, is a mixture of DNA from multiple sources, or is contaminated with interfering substances."

NRC I, 1992, p. 8

"For the complex DNA profile, there is no predominant or overarching standard interpretation method."

Peter Gill (Gill et al. 2012, report to the UK Forensic Science Regulator, p. 18)

"The limits of each DNA typing procedure should be understood, especially when the DNA sample is small, is a mixture of DNA from multiple sources…" **(NRC I, 1992, p. 8)**

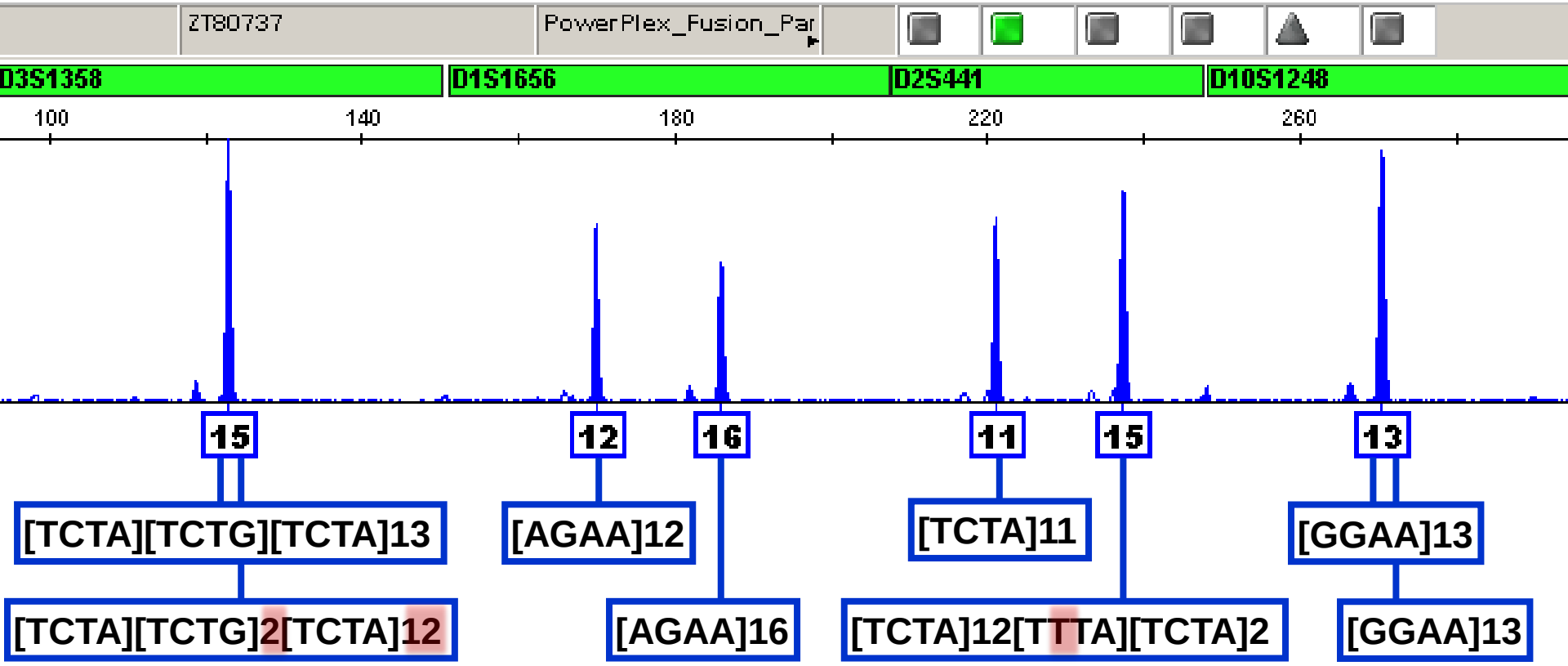
Butler, J.M. (2015) *Advanced Topics in Forensic DNA Typing: Interpretation* (Elsevier Academic Press: San Diego), pp. 159-182

Current Trends in Forensic DNA

- ***Faster** results:* Rapid DNA capabilities and new sample-to-answer integrated instruments
- ***Higher** sensitivity:* New assays lowering the limits of detection, which makes interpretation more challenging
- ***Higher** information content:* Next-generation sequencing (NGS) for more markers & STR allele information
- ***Stronger** conclusions:* Mixture interpretation with probabilistic genotyping models

Butler, J.M. (2015) The future of forensic DNA analysis. *Phil. Trans. R. Soc.* B 370: 20140252

Forensic STR Sequence Diversity



Sequence-Based Heterozygote: A locus that appears homozygous in length-based measurements (such as CE), but is heterozygous by sequence

Next Generation Sequencing (NGS)/ Massively Parallel Sequencing (MPS)

- **Higher information content** with sequence data
  - Expanded number of STR loci and other genetic markers such as SNPs and InDels
    - New markers may enable additional applications (e.g., biogeographical ancestry and phenotypic prediction)
  - **Deeper depth of information on STR alleles**
    - For example, eight different sequence versions of D12S391 alleles among 197 samples examined (Gelardi et al. 2014)

- **Significant challenges with BIG data**
  - STR allele nomenclature issues (ISFG DNA Commission - Parson et al. 2016)
  - Data storage (do you retain terabytes of data?)
  - Data analysis time will increase…
  - Privacy concerns with additional genomic information

True Sample Components

Sample Processing

DNA Data Obtained

Potential STR alleles

12 13 14 15 16 17 18 19

Total DNA amplified

4x

Genotype

13,17

female

13

17

1x

13 14

male

Mixture Ratio of Components

Potential Allele Overlap & Stacking

Number of Contributors
(sample components)

Validation
establishes variation and limits in the processes involved

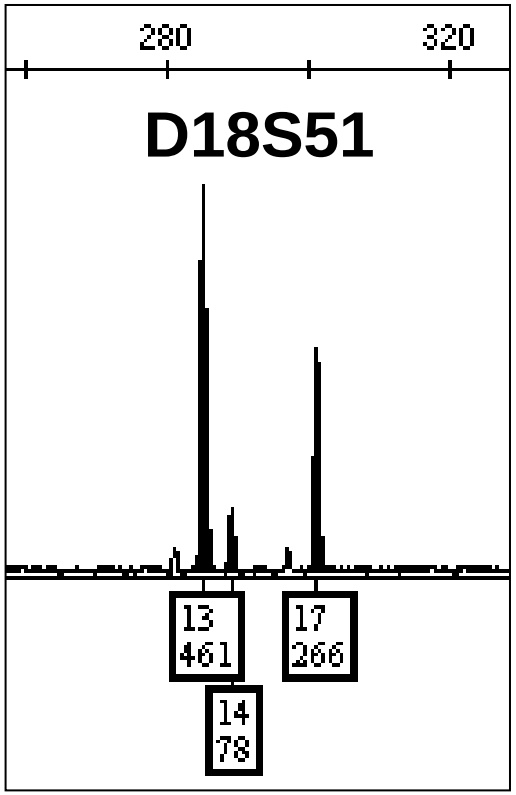
Extraction

PCR

CE Injection

CE Detection

portion of a CE electropherogram



Infer possible genotypes & determine sample components

From available data

Goal of Interpretation

Figure 6.2 in Butler (2015) *Advanced Topics in Forensic DNA Typing: Interpretation*

Updated Guidelines to Help with DNA Mixture Interpretation

Scientific Working Group on
DNA Analysis Methods
Interpretation Guidelines for
Autosomal STR Typing
by Forensic DNA Testing
Laboratories

Current guidelines are now 90 pages long and have examples

https://www.swgdam.org/publication

5 Reasons that DNA Results Are Becoming More Challenging to Interpret

1. **More sensitive DNA test results**
2. **More touch evidence samples** that are poor-quality, low-template, complex mixtures
3. **More options exist** for statistical approaches involving probabilistic genotyping software
4. **Many laboratories are not prepared** to cope with complex mixtures
5. **More loci being added** because of the large number of samples in DNA databases

http://www.cstl.nist.gov/strbase/pub_pres/Butler-DNA-interpretation-AAFS2015.pdf

Improved Sensitivity is a Two-Edged Sword

"As sensitivity of DNA typing improves, laboratories' abilities to examine smaller samples increases. This improved sensitivity is a two-edged sword. **With greater capabilities comes greater responsibilities to report meaningful results.** Given the possibility of DNA contamination and secondary or even tertiary transfer in some instances, **does the presence of a single cell (or even a few cells) in an evidentiary sample truly have meaning?**…"

Butler, J.M. (2015) *Advanced Topics in Forensic DNA Typing: Interpretation* (Elsevier Academic Press: San Diego), p. 458

More Touch Evidence Samples

https://www.ncjrs.gov/pdffiles1/nij/grants/222318.pdf

The DNA Field Experiment:
Cost-Effectiveness Analysis of the Use of DNA in the Investigation of High-Volume Crimes

John K. Roman
Shannon Reid
Jay Reid
Aaron Chalfin
William Adams
Carly Knight

Expanded DNA testing for burglary cases

NIJ April 2008 Research Report

http://www.nij.gov/journals/261/pages/dna-solves-property-crimes.aspx

NIJ Journal October 2008 (vol. 261, pp. 2-12)

- **More poor-quality samples are being submitted**
  - Samples with <100 pg of DNA submitted in Belgium:
    19% (2004) → 45% (2008)
    (Michel 2009 FSIGSS 2:542-543)
- AAFS 2014 presentations showed poor success rates
  - NYC (**A110**): **only 10% of >9,500 touch evidence swabs from 2007 to 2011 produced usable DNA results**
  - Allegheny County (**A114**): examined touch DNA items processed from 2008 to 2013 across different evidence types (e.g., 6 of 56 car door handles yielded "resolvable profiles")

New Options Exist for Statistical Analysis

- Increase in approaches to try and cope with potential allele dropout → number of **probabilistic genotyping** methods have grown since Balding & Buckleton 2009 article

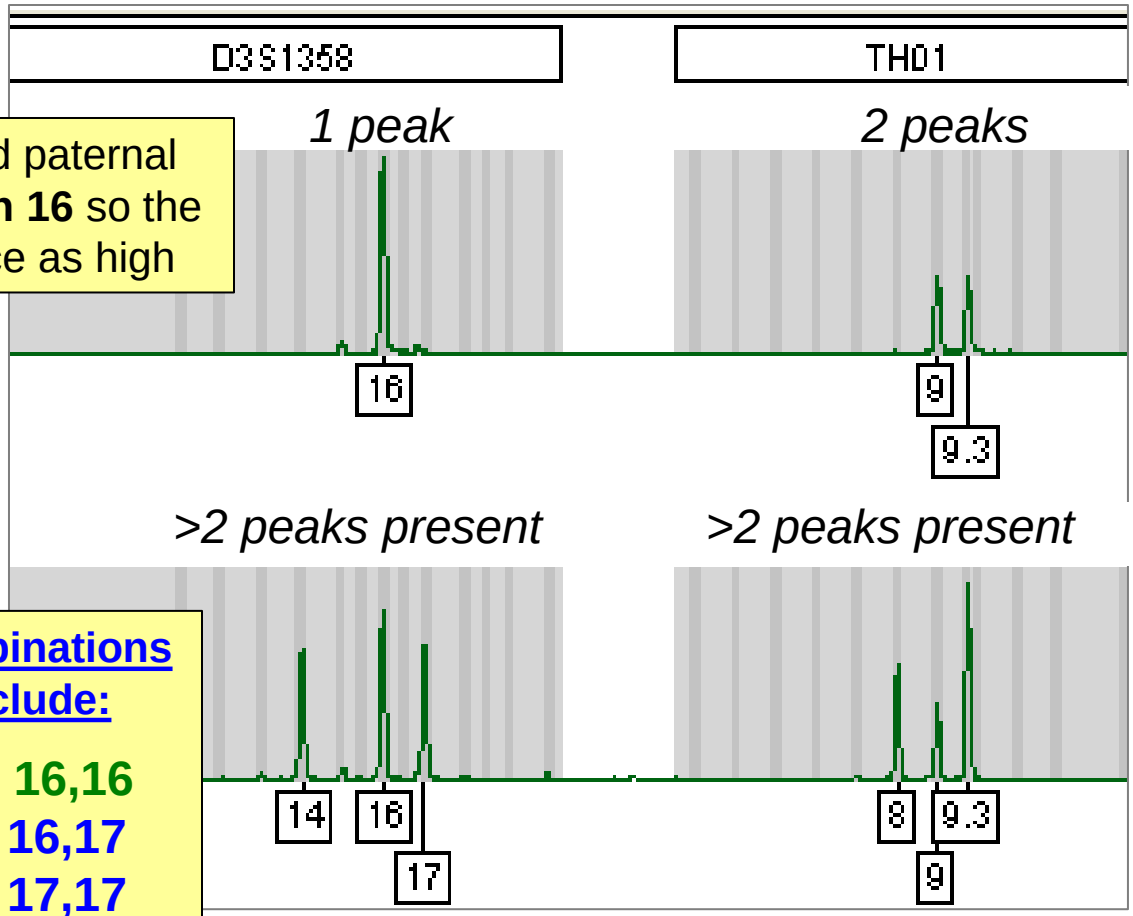
- Many possible choices for **probabilistic genotyping software** with commercial interests at stake

Balding, D.J. & Buckleton, J. (2009) Interpreting low template DNA profiles. *Forensic Sci. Int. Genet.* 4(1):1-10.

Gill P, Whitaker J, Flaxman C, Brown N, Buckleton J. (2000) An investigation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA. *Forensic Sci. Int.* 112(1):17-40.

Single-Source Sample vs Mixture Results

Maternal and paternal allele are **both 16** so the signal is twice as high



Single-Source

Mixture

Possible combinations at D3S1358 include:

14, 17 with 16,16
14,14 with 16,17
14,16 with 17,17

Multiple possible combinations could have given rise to the mixture observed here

Probabilistic Genotyping via Modeling Simulations

Mathematical Modeling of the Data

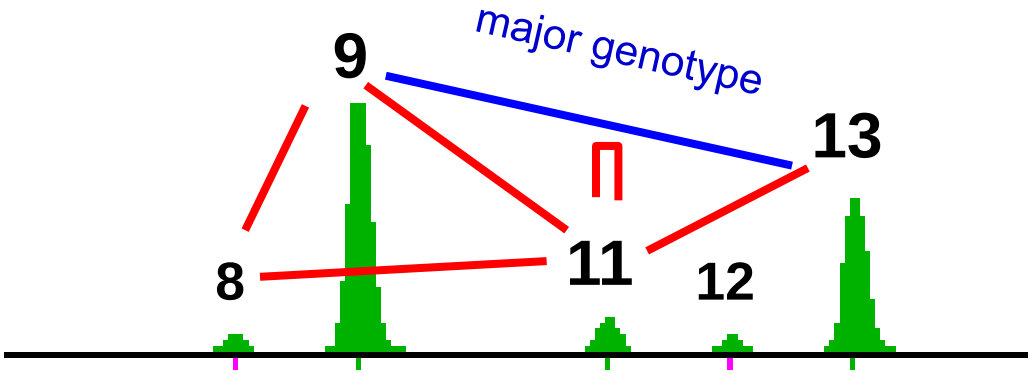
PHR, mix ratio, stutter, etc…

Typically thousands of simulations are performed

(MCMC)

Probable ***Genotypes*** to explain the mixture

D16S539



Minor Contributor Possible Genotypes	Probability
9,11	76%
11,11	15%
11,13	2%
8,11	2%
8,9	<1%
…	<1%

- Quantitative computer interpretation using numerous Markov Chain Monte Carlo (MCMC) simulations
- Models peak uncertainty and infers possible genotypes
- Results are presented as the Combined LR

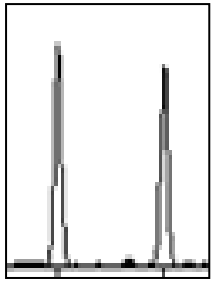
Math Analogy to DNA Evidence

$2 + 2 = 4$

$2x^2 + x = 10$

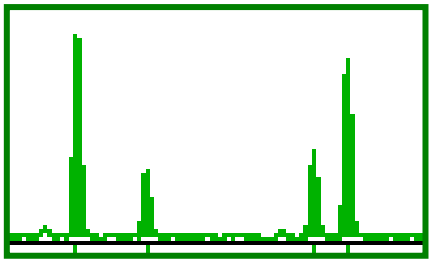
$$\int_{x=0}^{\infty} f(x)\,dx$$

Basic Arithmetic



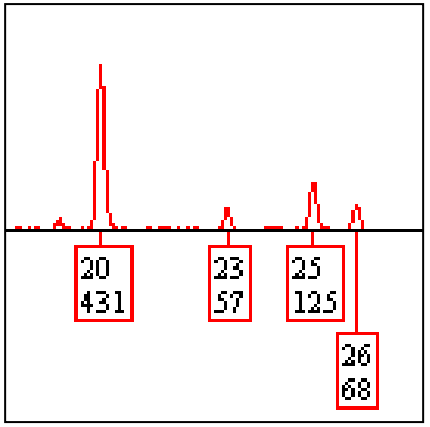
Single-Source DNA Profile
(DNA databasing)

Algebra



Sexual Assault Evidence
(2-person mixture with high-levels of DNA)

Calculus



Touch Evidence
(>2-person, low-level, complex mixtures perhaps involving relatives)

http://www.cstl.nist.gov/strbase/pub_pres/Butler-DNA-interpretation-AAFS2015.pdf

Many laboratories are not prepared
to cope with complex mixtures

- Have **appropriate validation studies** been performed to inform proper interpretation protocols? (curriculum & classroom instruction)

- Are **appropriately challenging proficiency tests** being given? (graded homework assignments)

- **Would we want to go into a calculus exam only having studied algebra and having completed homework assignments involving basic arithmetic?**

Perhaps We Should Slow Down with Some of the DNA Mixtures That We (Scientists and Lawyers) Are Taking On…

Poor Quality Conditions

Large Numbers of Contributors

The Future of Forensic DNA

is Similar to the Olympic Motto of

"Faster, Higher, Stronger"

Resources **Training** **Action**

National Commission on Forensic Science (NCFS):
www.justice.gov/ncfs

Organization of Scientific Area Committees (OSAC):
www.nist.gov/forensics/osac/index.cfm

NIST FORENSIC SCIENCES

www.nist.gov/forensics

+1-301-975-4049 john.butler@nist.gov